Atrial natriuretic peptide gene delivery attenuates gentamycin-induced nephrotoxicity in rats

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Abstract

Background. Atrial natriuretic peptide (ANP) is a cardiac hormone which exerts potent natriuretic and vasorelaxant activities. The aim of this study is to investigate potential protective effects of ANP gene delivery in gentamycin-induced nephrotoxicity.

Methods. Adenovirus (Ad.RSV-ANP) carrying the human ANP gene or carrying the LacZ gene (Ad.RSV-LacZ) under the control of the Rous sarcoma virus promoter were delivered intravenously on the first day of gentamycin administration. Sprague–Dawley rats were injected subcutaneously with gentamycin daily for 10 days.

Results. A single systemic injection of Ad.RSV-ANP at a dose of $1.2 \times 10^{10}$ pfu results in a significant increase in urine excretion, water intake, urinary sodium and potassium excretion. Adenovirus-mediated ANP gene delivery significantly increased renal blood flow, glomerular filtration rates and urine flow as well as attenuated the elevation of blood urea nitrogen levels. Histological evaluations revealed that ANP delivery attenuated gentamycin-induced renal tubular damage, cellular necrosis, and lumenal protein casts. The expression of human ANP mRNA was identified in rat kidney, heart, aorta and liver. Immunoreactive Atrial natriuretic peptide (ANP) is a 28-amino acid peptide hormone secreted predominantly by atrial cardiomyocytes [5]. ANP is released into the circulation in response to atrial distention caused by volume expansion, such as congestive heart failure and kidney disease [6]. ANP produces a number of biologic effects that influence blood pressure, bodily fluid excretion, and electrolyte balance through specific cell receptors. Three ANP receptors have been characterized [7]. Biologically active receptors (ANPRA or ANPR-B) are tightly coupled to guanylate cyclase and mediates biological activity through the second messenger cyclic guanosine monophosphate (cGMP). ANPR-C lacking guanylate cyclase activity functions as a clearance receptor to eliminate ANP from the circulation. Administration of exogenous ANP results in numerous physiological responses, including a rapid natriuresis and diuresis as well as a reduction in the arterial blood pressure [8]. The use of ANP as a therapeutic agent has been extensively explored. ANP has been shown

Introduction

Gentamycin is an aminoglycoside antibiotic that is commonly used in treating life-threatening Gram-negative bacterial infections. However, 30% of the patients treated with gentamycin for more than 7 days showed some sign of nephrotoxicity [1]. Serious complications resulting from gentamycin-induced nephrotoxicity are a limiting factor for its clinical usage [2]. Previous studies suggested that several factors contribute to the pathogenesis of gentamycin nephrotoxicity: lysosomal phospholipidosis due to inhibition of the activity of phospholipase A and C, hydroxyradical formation, inhibition of Na⁺-K⁺ ATPase, mitochondrial injury, and activation of the renin–angiotensin system [3,4]. However, the exact mechanism of gentamycin-induced nephrotoxicity is still unknown. A potential therapeutic approach to protect or reverse this antibiotic’s effects on renal tubular damage could have significant clinical applications. Gentamycin-induced acute renal failure is an excellent animal model for studying the pathogenesis of drug-induced acute renal failure and for developing therapeutic approaches to reduce or prevent its deleterious effects in humans.

Atrial natriuretic peptide (ANP) is a 28-amino acid peptide hormone secreted predominantly by atrial cardiomyocytes [5]. ANP is released into the circulation in response to atrial distention caused by volume expansion, such as congestive heart failure and kidney disease [6]. ANP produces a number of biologic effects that influence blood pressure, bodily fluid excretion, and electrolyte balance through specific cell receptors. Three ANP receptors have been characterized [7]. Biologically active receptors (ANPRA or ANPR-B) are tightly coupled to guanylate cyclase and mediates biological activity through the second messenger cyclic guanosine monophosphate (cGMP). ANPR-C lacking guanylate cyclase activity functions as a clearance receptor to eliminate ANP from the circulation. Administration of exogenous ANP results in numerous physiological responses, including a rapid natriuresis and diuresis as well as a reduction in the arterial blood pressure [8]. The use of ANP as a therapeutic agent has been extensively explored. ANP has been shown...
to cause blood pressure reduction in animals and hypertensive human subjects in either short or long term infusion [9]. Infusion of ANP was found to be effective in primary aldosteronism, glomerulonephritis, and renovascular hypertension by reducing blood pressure, lowering plasma aldosterone, and increasing natriuresis [10]. Our recent study indicated that adenovirus-mediated gene delivery of human ANP not only led to a prolonged blood pressure reduction but also resulted in protection against salt-induced cardiac hypertrophy and renal damage in hypertensive Dahl salt-sensitive rats [11].

In the present study, we evaluated the protective effects of human ANP gene delivery in the rat model of gentamycin-induced nephrotoxicity. The results show that systemic delivery of the human ANP gene in an adenovirus vector led to increased renal function and attenuation of renal lesions resulting from gentamycin administration. These findings suggest that ANP gene therapy may have potential applications in treating drug-induced nephrotoxicity.

Subjects and methods

Preparation of replication-deficient adenovirus vector Ad RSV-ANP

Plasmid RSV-ANP was constructed as previously described [12], in which the expression of human ANP cDNA was under the control of the Rous sarcoma virus long terminal repeat (RSV-LTR) and was followed by a SV40 poly A signal sequence. The transcription unit of RSV-ANP-poly A, including the RSV-LTR, the human ANP cDNA, and a Simian virus 40 poly A signal sequence, was released from the RSV-ANP plasmid by SalI digestion. Plasmid pAd.RSV-ANP was constructed by inserting the released fragment into the adenovirus shuttle vector pAdLink.1 at a SalI site. The pAd.RSV-ANP plasmid DNA was purified using a Qiagen plasmid DNA kit (Qiagen, Chatsworth, CA, USA). The purified DNA was sent to the Institute for Human Gene Therapy, Wistar Institute, Philadelphia to generate the adenovirus Ad.RSV-ANP harboring the RSV-ANP-poly A transcription unit. Final production of Ad.RSV-ANP and Ad.RSV-LacZ harboring the LacZ gene was carried out in this laboratory.

Animal treatment

Sprague–Dawley rats (male, 200–220 g body weight, Harlan Sprague Dawley, Inc., Indianapolis, IN, USA) were used in this experiment. Rats were housed at a constant room temperature with a 12 h light–dark cycle and had free access to tap water and rat chow. The rats were divided at random into four groups. Three groups received gentamycin sulfate (Sigma Chemical, St. Louis, MO, USA) subcutaneously at a dose of 50 mg/kg body weight and perfused with normal saline by cardiac puncture. Tissues were homogenized in normal saline with a Polytron (Brinkmann Instruments, Westbury, NY, USA). The homogenate was centrifuged at 600 g for 10 min. The supernatant was used as a probe for hybridization at 42 °C. The membrane was washed in 5× SSC twice at 42 °C and exposed to X-Omat film at −80 °C (Eastman Kodak Co., Rochester, NY, USA).

Serum and urine collection

At various time points after injection of adenoviral vectors, serum was collected and measured for blood urea nitrogen (BUN) levels using a modified urease-indophenol method [13]. Twenty-four-hour urine samples were collected on day 7 using metabolic cages. To eliminate food contamination in urine samples, rats were only given drinking water during urine collection. Urine samples were collected and centrifuged at 1000 g to remove particles. The volume was measured and the supernatant was used for further analysis.

Tissue preparation

At 10 days after gene delivery, one rat from each group was anesthetized intraperitoneally with pentobarbital at a dose of 50 mg/kg body weight and perfused with normal saline by cardiac puncture. Tissues were homogenized in normal saline with a Polytron (Brinkmann Instruments, Westbury, NY, USA). The homogenate was centrifuged at 600 g for 10 min. The supernatant was incubated in 0.5% sodium deoxycholate and then centrifuged at 10000 g for 30 min. Total protein in the supernatant was determined by Lowry’s method [14].

Radioimmunoassay (RIA) for human ANP

Immunoreactive human ANP levels were measured by direct RIA as previously described [12].

RIA for cGMP

Kidney tissues (100 mg) were homogenized in 1 ml 0.1 N HCl and the homogenate centrifuged at 12000 g for 30 min.
The supernatant was diluted to 1:100 and the procedure for assay of cGMP was conducted according to the general procedure as previously described [15]. Protein concentration was determined by Lowry's method [14].

Measurement of urine flow rate, glomerular filtration rate and renal blood flow

Renal function was determined at 10 days after human ANP gene delivery. Glomerular filtration rate (GFR) and renal plasma flow (RPF) were determined from the clearance of polyfructosan and para-aminohippuric acid, respectively [16]. Renal blood flow (RBF) was calculated from RPF and haematocrit. Timed urine volume was determined gravimetrically.

Morphological and histological investigations

Rats were anaesthetized with pentobarbital (50 mg/kg, i.p.) and kidneys were removed, cleaned, washed in saline, blotted and weighed. Sections of the kidney were preserved in 4% phosphate-buffered formaldehyde solution and embedded in paraffin. Five μm-thick sections were cut and stained with haematoxylin and eosin (H&E) and/or periodic acid-Schiff (PAS) and analysed microscopically and morphometrically. Morphological changes were scored as follows (Figure 1): (i) proximal tubule damage was found in parts of the outer cortex; (ii) proximal tubule damage was found throughout outer cortical nephrons; (iii) proximal tubule damage was found throughout outer and inner cortex; (iv) proximal tubule damage was found throughout the cortex and extended into the outer medulla. A score of zero (found only in controls) indicated no observable proximal tubule damage. Morphological evidence of proximal tubule damage included: swelling and disruption of cells, cell sloughing and tubular casts, and loss of PAS-positive apical brush border. All sections were evaluated independently by individuals without the prior knowledge of the group to which the rats belonged.

Statistical analysis

Group data are expressed as mean ± SEM. One- or two-way analysis of variance followed by a multiple means comparison test (Scheffe test) were used to compare the means of different groups. Differences were considered significant at a value of \( P < 0.05 \).

Results

Expression of human ANP mRNA in gentamycin-induced nephrotoxic rats

Human ANP mRNA in gentamycin-induced nephrotoxic rats after gene delivery was analysed by RT-PCR followed by Southern blot using specific oligonucleotide probes for human ANP. Total RNAs were prepared from heart, aorta, kidney, and liver at 4 days after intravenous injection of adenoviral vectors Ad.RSV-ANP or Ad.RSV-LacZ. ANP mRNA was detected in the kidney, heart, aorta and liver (Figure 2, upper panel, left). The expression of ANP mRNA was not detected in control rats receiving adenoviral vector Ad.RSV-LacZ (Figure 2, upper panel, right).

Similar levels of β-actin mRNA were detected in tissues of both experimental and control groups, confirming the integrity of RNA in these samples (Figure 2, lower panel). The results show that human ANP is expressed in tissues relevant to cardiovascular and renal function following gene transfer in gentamycin-induced nephrotoxic rats.

Immunoreactive human ANP levels after gene delivery

The levels of human ANP in gentamycin-induced nephrotoxic rats were analysed by a RIA specific for human ANP. Immunoreactive human ANP was detected in the heart (480.0 ± 21.5 ng/mg protein) and kidney (95.4 ± 9.2 ng/mg protein) at 4 days after gene delivery. Human ANP was also detected in the kidney...
ANP gene therapy in nephrotoxicity

Human and rat ANP are not immunologically identical and are distinguishable in the RIA.

Decreased blood urea nitrogen in rats receiving ANP gene delivery

Figure 4 shows blood urea nitrogen (BUN) levels in rats after gene delivery. BUN levels in the gentamycin-treated group with or without Ad.RSV-LacZ gene delivery began to rise 7 days after gentamycin administration. At 10 days post-gene delivery, there were two times higher levels of BUN in rats with gentamycin administration than the control group given saline (47.3 ± 0.3 and 44.7 ± 0.8 vs 24.4 ± 0.2 mg/100 ml serum, n = 5, P < 0.01). Although the BUN levels in rats injected with gentamycin and Ad.RSV-ANP (37.6 ± 0.9 mg/100 ml serum, n = 5) was significantly higher than the control group given saline, it was significantly lower than that of gentamycin-treated group with or without Ad.RSV-LacZ groups (P < 0.01). The results indicate that ANP gene delivery significantly attenuated BUN elevation in gentamycin-induced nephrotoxicity in rats.

Effects of ANP gene delivery on physiological parameters in gentamycin-induced nephrotoxicity in rats

Table 1 shows the results of physiological analysis of gentamycin-treated rats 7 days after gene delivery. There were no significant differences in all parameters between the control group and the gentamycin-treated groups with or without Ad.RSV-LacZ. However, ANP gene delivery caused a significant increase in urine volume (16.1 ± 1.3 vs 8.9 ± 1.1 ml/day/100 g body weight).

Figure 3. Time course of blood urea nitrogen (BUN) after gentamycin administration and gene delivery. Gentamycin was given subcutaneously, 80 mg/kg body weight daily, for 10 days. Adenovirus gene delivery was performed on the first day of gentamycin treatment. Data are expressed as mean ± SEM (n = 5). *P < 0.01 vs the gentamycin with and without Ad.RSV-LacZ groups; (○) control rats receiving saline; (●) rats receiving gentamycin alone; (▲) rats receiving gentamycin and Ad.RSV-LacZ. (▲) rats receiving gentamycin and Ad.RSV-ANP.

Figure 4. Time course of blood urea nitrogen (BUN) after gentamycin administration and gene delivery. Gentamycin was given subcutaneously, 80 mg/kg body weight daily, for 10 days. Adenovirus gene delivery was performed on the first day of gentamycin treatment. Data are expressed as mean ± SEM (n = 5). *P < 0.01 vs the gentamycin with and without Ad.RSV-LacZ groups; (○) control rats receiving saline; (●) rats receiving gentamycin alone; (▲) rats receiving gentamycin and Ad.RSV-LacZ. (▲) rats receiving gentamycin and Ad.RSV-ANP.
Table 1. Physiological analysis of gentamycin-induced nephrotoxic rats after ANP gene delivery

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control</th>
<th>Gentamycin</th>
<th>Gentamycin Ad.RSV-LacZ</th>
<th>Gentamycin Ad.RSV ANP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine volume (ml/day/100 g BW)</td>
<td>5.9 ± 0.4</td>
<td>9.6 ± 1.0</td>
<td>8.9 ± 1.1</td>
<td>16.1 ± 1.3*</td>
</tr>
<tr>
<td>Water intake (ml/day/100 g BW)</td>
<td>3.4 ± 0.8</td>
<td>7.4 ± 1.4</td>
<td>5.6 ± 1.0</td>
<td>17.5 ± 2.1*</td>
</tr>
<tr>
<td>Urinary Na output (μmol/day/100 g BW)</td>
<td>227 ± 11</td>
<td>142 ± 27</td>
<td>184 ± 17</td>
<td>307 ± 24*</td>
</tr>
<tr>
<td>Urinary K output (μmol/day/100 g BW)</td>
<td>590 ± 44</td>
<td>604 ± 23</td>
<td>632 ± 47</td>
<td>823 ± 56*</td>
</tr>
</tbody>
</table>

Gentamycin-induced nephrotoxicity in rats receiving adenoviral vectors Ad.RSV-cCHK or Ad.RSV-LacZ via the tail vein on the first day of gentamycin administration. Urine collection was performed 7 days after gene delivery. Values for each group are reported as mean ± SEM (n=8). Statistical significance among the four groups were determined by ANOVA. *P<0.05 vs gentamycin and Ad.RSV-LacZ group.

Effects of ANP gene delivery on renal function in gentamycin nephrotoxic rats

Table 2 shows the results of renal hemodynamics in gentamycin-induced nephrotoxic rats 10 days after gene delivery. ANP gene delivery caused a significant increase in urine flow (11.1 ± 0.3 ml/min/g kidney weight, n=4, P<0.01) as compared to the Ad.RSV-LacZ gene delivery group (5.0 ± 0.2 ml/min/g kidney weight, n=4), the gentamycin-treated group (4.4 ± 0.3 ml/min/g kidney weight, n=4), and the saline-injected control rats (5.7 ± 0.4 ml/min/g kidney weight, n=4). Similarly, rats injected with Ad.RSV-ANP induced significant increase in GFR (1.83 ± 0.05 vs 0.83 ± 0.04 ml/min/g kidney weight, n=4, P<0.01) and RBF (18.9 ± 0.9 vs 9.2 ± 0.6 ml/min/g kidney weight, n=4, P<0.01) when compared with rats receiving the control adenovirus Ad.RSV-LacZ.

Table 2. Effects of human ANP gene delivery on renal function of gentamycin nephrotoxic rats

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control</th>
<th>Gentamycin</th>
<th>Gentamycin Ad.RSV-LacZ</th>
<th>Gentamycin Ad.RSV ANP</th>
</tr>
</thead>
<tbody>
<tr>
<td>UF (ml/min/g KW)</td>
<td>5.7 ± 0.4</td>
<td>4.4 ± 0.3</td>
<td>5.0 ± 0.2</td>
<td>11.1 ± 0.3*</td>
</tr>
<tr>
<td>GFR (ml/min/g KW)</td>
<td>0.94 ± 0.06</td>
<td>0.73 ± 0.05</td>
<td>0.83 ± 0.04</td>
<td>1.87 ± 0.05*</td>
</tr>
<tr>
<td>RBF (ml/min/g KW)</td>
<td>11.1 ± 0.9</td>
<td>8.7 ± 0.8</td>
<td>9.2 ± 0.6</td>
<td>18.9 ± 0.9*</td>
</tr>
</tbody>
</table>

Gentamycin-induced nephrotoxicity in rats receiving adenoviral vectors Ad.RSV-ANP or Ad.RSV-LacZ via the tail vein on the first day of gentamycin administration. Renal function studies were performed 10 days after gene delivery. UF, urine flow; GFR, glomerular filtration rate; RBF, renal blood flow; KW, kidney weight. Values for each group are reported as mean ± SEM (n=4). Statistical significance among the four groups were determined by ANOVA. *P<0.01 vs gentamycin and Ad.RSV-LacZ group.
saline (1.3±0.1 and 1.3±0.1 vs 2.8±0.1 pmol/mg protein, n=4, P<0.01). However, a significant increase of cGMP in the kidney was observed in the Ad.RSV-ANP group (4.0±0.1 pmol/mg protein, n=4, P<0.01) compared to the control Ad.RSV-LacZ group or control with saline.

**Effects of ANP gene delivery on kidney morphology**

Figure 6 shows the morphology of kidney stained with H&E and examined by light-microscopy. The fixation, embedding and staining of non-treated control animals (Figure 6A) resulted in well preserved kidney morphology in both the cortex and medulla. A widespread tubular dilation and damage were observed in the renal cortex of gentamycin-treated rats either alone or followed by Ad.RSV-LacZ (Figure 6B and C). Most proximal tubules were damaged and many were either dilated or filled with necrotic cells. Distal tubules exhibited less damage and collecting ducts appeared relatively normal. Cortical renal tubular lumens were often filled with protein casts. Medullary tubules did not appear damaged in structure, but protein casts often filled the lumens, particularly in the outer medulla. In the ANP gene-treated group (Ad.RSV-ANP), swelling of proximal tubular cells was seen, but fewer dilated renal tubules were present and frank cellular necrosis was rare (Figure 6D).

Figure 7 shows the morphology of kidney stained by PAS for carbohydrates in brush borders and basement membranes. The morphology of the kidney in control rats (Figure 7A) without gentamycin treatment was well preserved in both the cortex and medulla. Proximal tubule brush border throughout cortex and outer medulla stained prominently with PAS, as did basement membrane in renal glomeruli and proximal tubules. In the renal cortex of rats injected with gentamycin alone or gentamycin and Ad.RSV-LacZ, there were dilated proximal tubules generally lacking of brush border. Lumenal casts were generally PAS-positive, most probably derived from sloughed brush border glycoprotein and reabsorption droplets from damaged proximal tubule cells (Figure 7B and C). The ANP gene-treated group (Figure 7D) exhibited less damage than either gentamycin alone or gentamycin with Ad.RSV-LacZ group. Although some tubular damage persisted, evidence of preservation and regeneration of proximal tubules included: intense PAS staining of the apical brush border in many tubules and fewer protein casts throughout the kidneys.

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*Fig. 6.* Histological sections of kidney cortex stained with haematoxylin and eosin. (A) Control rat, saline injection. Normal cortical morphology. (B) Rat, injected with gentamycin daily for 10 days. Note damaged proximal tubule; apical swelling, tubular dilation, and tubular casts. (C) Rat, injected with gentamycin daily for 10 days, receiving control gene Ad.RSV-LacZ on day 1. Damage comparable to gentamycin alone. (D) Rat, injected with gentamycin daily for 10 days, receiving Ad.RSV-ANP on day 1. Note recovery of many proximal tubules, although some damage remains. (Magnification ×100).
Fig. 7. Histological sections of kidney cortex stained with PAS. (A) Control rat, given saline. Note prominent PAS staining of proximal tubular brush border. (B) Rat injected with gentamycin alone. Note dilated tubules and absence of brush border in proximal tubules. Note also some PAS positive protein casts. (C) Rat injected with gentamycin for 10 days, receiving control gene, Ad.RSV-LacZ. Damage comparable to rats seen with gentamycin alone. (D) Sprague–Dawley rat, injected with gentamycin daily for 10 days, receiving Ad.RSV-ANP, the human tissue kallikrein gene construct. Note: presence of brush border in several proximal tubules, although some damaged tubules are still evident. (Magnification $\times 100$).

Figure 8 shows the effect of ANP gene delivery on morphological changes of the kidney assessed by PAS-stained sections. The quantitative evaluation of renal tubular damage was scored as described in Figure 1. Gentamycin-treated animals with or without Ad.RSV-LacZ had the most extensive damage (Score: gentamycin, 3.0 $\pm$ 0.3; gentamycin and Ad.RSV-LacZ, 2.7 $\pm$ 0.3, $n=8$) and there were no differences between these two groups. Although the rats receiving gentamycin and Ad.RSV-ANP had some tubular damage (Score: 1.3 $\pm$ 0.1, $n=8$), the damage was significantly less than in two control groups ($P<0.01$). The results indicate that ANP gene delivery significantly attenuated tubular damage induced by gentamycin.

Discussion

In this study, we have shown that adenovirus-mediated delivery of the human ANP gene via intravenous injection displayed protective effects on gentamycin-induced nephrotoxicity in rats. Histologically, proximal tubular damage was diminished. ANP gene delivery significantly attenuated the elevation of BUN levels. In addition, ANP gene delivery also enhanced renal function and increased cGMP levels in the kidney. The expression of ANP mRNA was identified in tissues relevant to cardiovascular and renal function. These findings suggest a potential application of ANP gene
therapy for the treatment of drug-induced renal damages.

The effect of ANP on ischaemic or nephrotoxic animal models of acute renal failure has been shown to cause improvement in renal function as well as renal damage [9,10]. In addition, our recent studies showed that adenovirus-mediated gene delivery of ANP alleviated renal damage in Dahl-salt sensitive rats fed a high salt diet [11]. These findings show that it is possible to maintain a continuous supply of ANP by gene delivery and to use this technique for studying its potential effects on renal function and renal injury. A significant increase in urine flow, GFR and RBF were observed in gentamycin-treated rats after ANP gene delivery. These results show that systemic delivery of the ANP gene had a protective effect on renal function in this rat model of acute renal failure. These findings are consistent with our recent studies that ANP gene delivery enhanced renal function in two kidney, one clip Goldblatt hypertensive rats with renal vascular hypertension (Yayama K., Wang C., Chao L., Chao J. Human atrial natriuretic peptide gene delivery attenuates hypertension, cardiac hypertrophy and enhances renal function in Goldblatt hypertensive rats. Kidney Int 1998; Submitted for publication). ANP increases GFR by dilating afferent and constricting efferent arterioles, leading to increased hydraulic pressure in glomerular capillaries and increasing the hydraulic pressure gradient from capillary lumen to Bowman’s space [17]. In addition, direct actions of ANP on glomerular mesangial cells lead to relaxation that may, in some instances, result in increases in the glomerular ultrafiltration coefficient.

Gentamycin is filtered through the glomerular basement membranes and is taken up via pinocytosis into proximal tubular cells [18]. Renal injury from aminoglycoside administration results from the accumulation of this drug in proximal tubules and interfere with cellular energetics [3]. Morphological analysis by quantitative evaluation of PAS-stained sections showed significant suppression of renal injury after human ANP gene delivery. A previous study showed peak nephrotoxic damage on renal function around 10 days after gentamycin treatment [19]. Therefore in this study, we evaluated morphological and physiological changes at 10 days after gentamycin administration. Although ANP could not completely protect the renal damage, it significantly attenuated the renal proximal tubular damage compared to gentamycin-treated rats with or without Ad.RSV-LacZ. We also noticed the occurrence of mitotic figures in the renal proximal tubules of ANP gene delivery group but not in the control group receiving Ad.RSV-LacZ (data not shown). However, using different protocol, mitotic figures could be observed in the kidney of rats receiving 2 days of gentamycin treatment [20]. Further studies are needed to analyse ANP-mediated protective effects by gene delivery in gentamycin-induced renal lesions.

There are several possible mechanisms to explain the attenuation of gentamycin-induced renal damage by ANP gene delivery. The most likely possibility may be attributed to the enhanced renal function. Increased GFR and RBF can reduce the accumulation of gentamycin in renal proximal tubules and thus reduce the possibility of cell necrosis. Because of the striking increase in sodium and potassium excretion, a direct effect of ANP on proximal tubule might be considered. Although there is some evidence that ANP alters solute transport in the proximal tubule, it has been difficult to demonstrate a direct action of ANP in proximal tubule cells [21]. Thus, it remains unclear whether ANP can alter solute transport in the proximal tubule by acting on the epithelial cells. It is possible that the effects of ANP on proximal tubule transport in vivo are mediated by changes in transepithelial driving forces governing solute transport, or that other cells, such as those of blood vessels, juxtaglomerular apparatus, or glomeruli produce mediators in response to ANP which in turn alters transport properties of the proximal tubules. In addition, ANP interacts at several levels with the renin–angiotensin–aldosterone system. ANP has a direct effect on the adrenal gland to inhibit the production of aldosterone and also on juxtaglomerular cells to reduce renin release [22] and it attenuates the vasoconstrictor effects of angiotensin II [23]. Based on these findings, it seems likely that the ability to attenuate gentamycin-induced renal damage by ANP gene delivery may be due, at least in part, to reduced accumulation of gentamycin by enhanced renal function and altering transport properties in renal proximal tubules. In addition, the inhibition of renin–angiotensin–aldosterone systems might also play a role in the protection against renal nephrotoxicity.

In this study, the level of cGMP in kidney was significantly decreased in gentamycin-treated rats and renal cGMP levels increased markedly after ANP gene delivery. These findings suggest that increased renal function following ANP gene delivery is mediated by increased cGMP levels. The effect of ANP on renal function has been extensively investigated. ANP infusion has been shown to improve renal function in experimental animal models [24]. In this study, we showed that adenovirus-mediated gene delivery results in expression of ANP in rat kidney. However, cellular localization of ANP and its mRNA in rat kidney has yet to be identified. Binding of ANP to the receptor may trigger activation of guanylate cyclase and mediate biological activity through the second messenger cGMP. The influence of other potential second messengers such as cAMP or eicosanoids in triggering ANP effects remains to be elucidated.

In this study, we employed somatic gene delivery approach to explore the effects of ANP on gentamycin-induced nephrotoxicity in rats. There are many advantages of gene delivery approaches over protein or peptide infusion. First, one single injection of the gene encoding ANP resulted in a long-term expression of the gene product for several weeks [12]. Two, gene delivery is simple to administer and thus eliminates complicated manipulation of surgical procedures for peptide infusion. Three, gene delivery allows long-term study of the physiological function of a specific gene...
product. Fourth, gene delivery is inexpensive as compared to using transgenic animal models. A previous study examined the effect of acute infusion of ANP on gentamycin-induced acute renal failure [25]. They showed that GFR was decreased following gentamycin application while ANP infusion markedly increased GFR to the basal level. The effects of ANP on impaired renal function were immediately blunted after cessation of ANP infusion. In this study, we observed that a single intravenous injection of the ANP gene resulted in enhanced renal function as well as protection against renal injuries induced by gentamycin. Similar findings were reported in our previous study that adenovirus-mediated gene delivery of human tissue kallikrein resulted in significant increases in RBF and GFR as well as protection against gentamycin-induced [16]. These results suggest that delivery of the human ANP or tissue kallikrein gene might reduce accumulation of gentamycin in the kidney and thus prevent tubular damages by enhanced renal function.

In conclusion, adenovirus-mediated human ANP gene delivery has protective effects on nephrotoxicity in gentamycin-treated rats. Gentamycin-induced nephrotoxicity can be treated with drugs or dietary supplements such as fish oil or calcium, but these treatments require daily administration [26]. This study shows that a single injection of ANP gene can attenuate renal injury as well as enhance renal function. New vectors for gene therapy are currently under development to achieve high level and long-term gene expression. Further technical improvements in delivery methodologies may lead to future clinical application of gene therapy in treating drug-induced renal injuries.

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